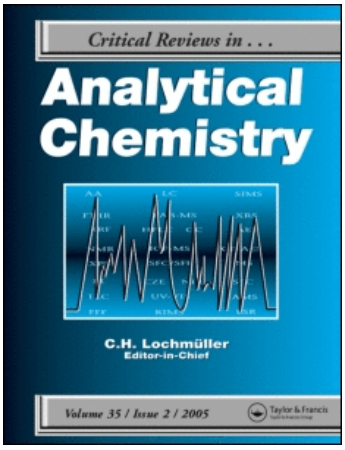

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Chemiluminescence-Based (Bio)Sensors — An Overview

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ABSTRACT: This review discusses the advances in the design of chemiluminescence-based sensors and biosensors with particular emphasis on their classification. Several up-to-date applications are presented.

KEY WORDS: chemiluminescence, sensor, biosensor, design.

I. INTRODUCTION

Chemiluminescence (CL) is well known for its high sensitivity (10^{-6} – 10^{-15} g), and for the lower detection limits (10^{-18} g) that can be obtained. To improve the precision of analytical information, CL-based sensors and biosensors were developed.

A lack in the selectivity of CL is recorded especially when it is used directly as an usual spectrometric technique. Bakker[1] compared the selectivity of optical sensors with the selectivity of potentiometric sensors. The utilization of CL-based sensors significantly increased the selectivity of the CL itself due to the selectivity of the reaction involved prior to the CL one. The best selectivity can be obtained by prior utilization of an enzymatic or immunosensor reactions.[2,3] For complex matrices, separation techniques are necessary before CL detection.[4]

Due to their electronic structure, some metals (e.g., Ru, Ce) can easily be used for direct CL generation.[5,6] However, the CL of luminol is favored by an alkaline medium in the presence of certain inorganic ions or molecules (e.g., MnO_4^-, I_2, $[Fe(CN)_6]^{3-}$, Cu^{2+}, OCl^-). It was discovered that the unsaturated complex of Cu(II) with proteins had a much stronger catalytic effect on the luminol–H_2O_2 CL reaction than Cu(II) alone; this principle was also used for the proteins assay.[7]

Significant advances in the design and applications of CL-based sensors and biosensors were recorded in the last few years.[8] The most utilized sensor type was flow-through (bio)chemical sensors-based CL.[9] The reliability of these sensors made them suitable for utilization as detectors in FIA systems.[10]

The instrumentation involved in CL measurements is simple. For CL-based sensors, the performances of a multifunctional

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optical-fiber spectrometer were tested; good reproducibility and precision were recorded.[11]

II. THE DESIGN OF CHEMILUMINESCENCE-BASED (BIO)SENSORS

For CL-based (bio)sensors the most utilized design is a flow-through (bio)sensor one. A rigorous description of these was given by Valcarcel et al.[9] and by Coulet and Blum.[12]

The base of CL-based sensors construction is the electrochemical sensor that forms a compound at its interface, which is measured using a flow of luminol,[13] ruthenium complex,[14] or riboflavin phosphate[15] by CL generation. The best sensitivity and selectivity is assured by utilization for H_2O_2 generation of biosensors,[14] microbial sensors,[16] or immunosensors.[17]

Kearney et al.[18] improved the sensitivity of H_2O_2 generation using platinum electrodes pretreated by washing with 2 mol/l HNO_3 solution for 180 s, then passing a 0.18 mol/l H_2O_2 solution for 60 s. The luminol solutions were injected into the system in a chloride carrier of pH 12.5 at a flow rate of 4.5 ml/min. The method proved to have a very good sensitivity for the system: the linearity of the calibration graph is in the 1 nmol/l to 100 μmol/l H_2O_2 concentration range with a low detection limit (1 nmol/l). The sensitivity of the CL generation was increased by the immobilization of a *tris*(2,2′-bi-pyridyl)ruthenium (II) complex[19] on the electrochemical sensor surface.

III. CHEMILUMINESCENCE-BASED SENSOR AND BIOSENSOR TYPES

A. Chemiluminescence-Based Sensors

1. Inorganic Substances Assay

a. Chlorine

The sensor proposed for chlorine assay[20] consisted of a Pyrex tube, packed with the uranine [fluoresceine disodium] complex immobilized on IRA-93 anion-exchange resin, and a photomultiplier tube placed close to the Pyrex tube. It was used for the monitoring of the concentration of free chlorine (as HClO) in tapwater, up to1 mmol/l, with a detection limit of 2 μmol/l. The coefficient of variation (n = 10) obtained for the free chlorine assay is 1.6%, for a concentration of 10 μmol/l. The main disadvantage is the short lifetime of the sensor.

B. Copper

The copper CL-based sensor[13] comprised an anion-exchange column having luminol and cyanide permanently co-immobilized on the resin, while Cu was temporarily retained by electrochemical preconcentration on a Au electrode placed in an anodic stripping voltammetric cell. Injection of 0.1 mol/l NaOH through the column elute the reagents that then reacted with Cu stripped from the electrode to produce a chemiluminescent signal. The response was linear in the 0.01 to 10 μg/l Cu(II) solution concentration range, with a detection limit of 8 ng/l. The RSD value at 40 ng/l concentration level was 7.4%, for the assay of Cu(II) in natural waters and human serum.

C. Hydrazine

For the assay of hydrazine and its monomethyl and dimethyl derivatives, two types of experiments with CL-based sensors are proposed, according to *ex situ* and *in situ* types of analysis.[21] For the *ex situ* method an additional device is necessary: the sample diluted in air was delivered at 1 ml/min by diffusion through a cellulose membrane to a chamber containing *tris*(2,2′-bipyridyl) ruthenium complex in 0.1 mol/l phosphate buffer; the analytes were oxidized using a Pt working electrode. The detection limits are of ng/l concentration level for both *in situ* and *ex situ* analyses.

D. NO_2

Three types of CL-based sensors are proposed for NO_2 assay. Heilmann et al.[22] proposed a lead phthalocyanine thin film sensor. The sensor chip consisted of a thin film layer (200 nm) prepared by vacuum sublimation of purified powder of lead phthalocyanine and comb-like Au electrodes. The best sensitivities can be achieved by utilization of the CL-based sensors proposed by Spicer et al.[23]: the first one is based on the reduction of NO_2 to NO followed by the detection of NO by the CL produced from its reaction with O_3, while the second one is based on the detection of CL produced from the reaction of NO_2 with luminol solution. The working concentration range for the O_3 based CL sensor (0 to 800 μg/l NO_2) is larger than the working concentration range obtained by NO_2-based CL sensor (0 to 50 μg/l). The main disadvantage of these types of CL-based sensors is nonselectivity over a lot of substances (e.g., nitrous acid).

E. O_2

The system proposed for the O_2 assay[24] incorporates a solid-phase reagent across which the air under test is pumped, positioned below a photomultiplier tube for measurement of the resulting CL. Of the hydrogel or polymeric sorbents investigated, a fluoropolyol incorporating luminol, KOH, and iron (III) sulfate as catalyst gave the best results for O_2 assay, having a detection limit of 2.4 mg/l.

F. H_2O_2

The assay of H_2O_2 can be done using CL-based sensors in the presence of luminol and Co(II)[25,26] or Cu(II)[25] ions. Using Co(II) and Cu(II) foils,[25] H_2O_2 can be determined in the 0.1 to 200 μmol/l and 5 to 200 μmol/l concentration range, respectively. To improve the selectivity, a gas dialysis cell was used. By immobilization of luminol and Co(II) on a strongly basic anion-exchange resin and a weakly acid cation-exchange resin, H_2O_2 can be determined in the 40 nmol/l to 10 μmol/l concentration range with a limit of detection of 12 nmol/l. The high sensitivity of the last system[26] made the assay of glucose in blood possible; the selectivity is improved by utilization of a packed bed reactor with immobilizatized glucose oxidase.

G. S^{2-}

The flow-through sensor proposed for S^{2-} assay[15] consists of a glass column packed with a homogeneous bed of resin with permanganate and another resin with riboflavin phosphate. CL was transduced to an electrical signal with a photomultiplier tube. S^{2-} can be assayed from beer and wine on 0.1 to 100 mg/l concentration range with a detection limit of 0.06 mg/l, with a RSD of 3.7% for 1.0 mg/l of sulfite.

H. $S_2O_8^{2-}$

A micro-ring electrode is proposed for the $S_2O_8^{2-}$ assay.[27] A gold-coated optical fiber was polished to a flat surface, such that the gold formed a micro-ring electrode around the optical fiber. This-(2,2′-bipyridyl)ruthenium (II) was used as reagent for the CL generation. The detection limit was 4 nmol/l.

1. Organic Substances Assay

a. Adrenaline

A flow-through CL sensor based on Mn(III)-tetrakis-(4-sulfonatophenyl) porphyrin immobilized on a dioctadecyldimethylammonium chloride bilayer mem-

brane incorporated into a PVC blended film was developed for the determination of adrenaline.[28] The calibration graph was linear from 3 μmol/l to 0.3 mmol/l of adrenaline solution. The sample assay rate was 40 samples/h, and the RSD (n = 10) for 50 μmol/l was found to be 1.0%.

b. Ascorbic Acid

Three sensors based on luminol and different cations immobilization on a resin are proposed for ascorbic acid assay, as follows: (1) D-201 type anion-exchange resin containing luminol and permanganate immobilized;[29] (2) D-201 × 7 anion-exchange resin was used for immobilization of luminol and 732 cation-exchange resin (Na form) was used for Fe(II) immobilization;[30] (3) amberlyst A-27 anion-exchange resin continuing immobilized luminol and potassium ferricyanide.[31] Using these types of flow-through sensors, ascorbic acid can be assayed on: (1) 10 μg/l to 4 mg/l; (2) 1 nmol/l to 1μmol/l, and (3) 0.01 to 08 μg/ml concentrations ranges, with the following detection limits: (1) 5 μg/l; (2) 0.4 nmol/l, and (3) 5.5 ng/ml, respectively, while the first proposed sensor is free of interferences, Cu(II), thiourea, uric acid, and vitamin B_1 seriously interfere with the third sensor.

c. Dimethyl Sulfide

A high-speed sensor for the assay of dimethyl sulfide in the marine troposphere, based on its CL reaction with F_2 is proposed.[32] Sample air and F_2, in He were introduced at opposite ends of a reaction cell with a window at one end. The production of vibrationally excited HF and electronically excited fluorohydrocarbon (HCF) was monitored with a photomultiplier tube. Dimethyl sulfide can be determined on the 0 to 1200 pptv (parts per trillion by volume) concentration range, with a 4 pptv detection limit.

d. Ethanol

All sensors proposed for ethanol assay comprising a δ-Al_2O_3 layer, which can be coated with α-Al_2O_3[33,34] or with Pt thin film.[35,36] The limits of detection are on mg/l magnitude order when Pt is used for coating and on μg/l magnitude order when α-Al_2O_3 is used for coating. The CL reaction is based on ethanol oxidation as favored by these sensors. The presence of water, even in trace amounts reduced the sensitivity of the CL-based sensor.

e. Oxalate

An electrode based on *tris*-(2,2′-bipyridyl)ruthenium (II) complex immobilized in a Nafion film is proposed for CL assay of oxalate.[37] The limit of detection is 1 μmol/l, and the working concentration range is over four orders of magnitude.

f. Trichloroethylene

An optical-fiber CL-based sensor is described for trichloroethylene assay.[38] The sensor consisted of a glass fiber bundle and a transducer consisting of three components: (1) a gas-permeable membrane to separate trichloroethylene from water, (2) H_2SO_4-$NaNO_3$ mixture as oxidizing agent, and (3) luminol solution. The assay of trichloroethylene can be done on 0.05 to 0.6 μg/ml concentration range with a detection limit of 0.03 μg/ml.

g. Uric Acid

For the assay of uric acid a sensor based on $KMnO_4$-octylphenyl polyglycol ether is proposed.[39] Uric acid can be assayed directly, in urine in the 0.10 to 600 μg/ml concentration range with a detection limit of 55 ng/ml. The system is free of interferences.

B. Chemiluminescence-Based Biosensors

1. Inorganic Substances Assay

a. Antimonite and Arsenite

A highly sensitive and selective sensing system for antimonite and arsenite was developed based on genetically engineered bacteria harboring the plasmid Pbgd 23.[40] Ars R used in sensor design has a high specificity for antimonite/arsenite, thus confering to this sensing system high sensitivity and also specificity. Ars R, the gene encoding for the Ars R regulatory protein of the ars operon, is fused to laCZ, the gene encoding for the receptor enzyme β-galactosidase. The detection limit achieved with this sensor is of 10^{-15} mol/l magnitude order.

b. H_2O_2

Horseradish peroxidase (HRP)-based biosensors are proposed for the assay of H_2O_2.[41,42] HRP was immobilized by microencapsulation in sol-gel crystals derived from tetramethyl orthosilicate.[42] H_2O_2 can be assayed in the 0.1 to 3 nmol/l concentration range with a detection limit of 10^{-4} mol/l magnitude order.

c. S^{2-}

An optical fiber biosensor was developed for the determination of sulfite in foods based on reaction of sulfite with O_2 in the presence of immobilized sulfite oxidase to yield H_2O_2, which was then detected by a CL reaction using luminol in the presence of peroxidase.[43] S^{2-} can be determined in food using this CL-based biosensor on 1 to 100 μmol/l concentration range with a 0.5 μmol/l detection limit.

2. Organic Substances Assay

A. L-Alanine

A packed bed flow microreactor containing alanine aminotransferase immobilized on sieved porous glass beads was combined with a CL detector.[44] To catalyze the indicated reaction between luminol and H_2O_2, Co(II) and immobilized peroxidase from *Arthromyces ramosus* (ARP) were used in a fiber optic cell. L-Alanine was determined from cell cultivation media in the 2 to 500 μmol/l concentration range with a limit of detection of 1 μmol/l using Co(II), and in the 5 to 800 μmol/l concentration range with a limit of detection of 2 μmol/l when ARP was used.

b. Choline

The detection of choline using a CL-based biosensor is based on the immobilization on a polymer[45] or nylon[46] of choline oxidase and fungal peroxidase. The calibration graphs were linear in 0.1 to 1 μmol/l concentration range with a limit of detection of 1 μmol/l.

c. Glucose

Three types of CL-based biosensors are described for glucose assay. The first type was based on utilization of glucose oxidase for enzymatic reaction, in coupling with luminol for CL reaction.[47,48] The working concentration range is on mmol/l magnitude order, with a detection limit of 1 μmol/l. The second type was based on the utilization of glucose dehydrogenase enzyme for enzymatic reaction, in coupling with *tris*(2,2′-bipyridyl) ruthenium (II) complex for CL reaction.[49] This sensor can be used on 10 to 2500 μmol/l concentration range. There are

a lot of interferences like NADH, oxalate, proline, and tripropylamine. However, gluconic acid and NAD^+ do not interfere. A CL-based microbial sensor is also proposed for glucose assay.[16] For sensor construction, the microbe cells were introduced on a chitosan gel that was applied to the pH-sensitive field-effect transitors (FET) surface. Glucose can be assayed using this sensor in 0.1 to 1 mmol/l concentration range.

d. L-Lactate

Enzyme-modified silica and graphite pastes were used to construct CL-based biosensors for L-lactate.[50] L-lactate oxidase was coupled with luminol/Na_2CO_3 (pH = 9.2) to generate the CL. The system is very sensitive and selective when it is used in clinical analysis.

e. NADH

A regenerable electrogenerated CL-based biosensor for NADH based on dehydrogenase and *tris*-(2,2′-bipyridyl) ruthenium (II) complex immobilized on Eastman AQ 55S and Nafion cation-exchange polymer films is proposed.[14] The working concentration range is 0.2 to 5 nmol/l. Blume et al.[51] proposed a bioluminescence-based biosensor for the assay of NADH. A bioactive layer was designed using a commercial preactivated polyamide membrane to which bacterial luciferase and oxidoreductase were covalently bound. The calibration graph was linear in the 10 pmol/l to 0.5 nmol/l concentration range.

f. Xanthine and Hypoxanthine

CL-based enzyme sensors for xanthine and hypoxanthine assay are developed by covalent immobilization of microbial peroxidase (from *Thromyces ramosus*) or xanthine oxidase, on preactivated nylon membranes.[52] CL was produced based on the luminol-H_2O_2 reaction. The detection limit was of 5 μmol/l.

IV. CONCLUSIONS

CL-based(bio)sensors improved the sensitivity and selectivity of the CL itself by utilization of an enzymatic and antigen-antibody reaction that are able to form a substance active in CL reaction. The sensitivity is increased by utilization of a very sensitive reaction for the CL active substance formation, as well as by improving of the sensitivity of CL reaction (utilization of new reagents and new molecules and ions that can favor the reaction).

Although the CL-based (bio)sensors may assure the best sensitivity from all types of (bio)sensors, their selectivity is not good enough, even if the enzymatic and immunological reactions are involved. It is essential in the future to improve the selectivity of CL reaction by the use of new reagents. The reliability of analytical information made these sensors capable for utilization as detectors in FIA systems. They can also be used successfully for the determination of trace inorganic and organic substances.

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